JOURNAL OF VIROLOGY, Nov. 1995, p. 7269–7273 Vol. 69, No. 11
0022-538X/95/$04.00+0

A Novel Vβ 2-Specific Endogenous Mouse Mammary Tumor Virus Which Is Capable of Producing a Milk-Borne Exogenous Virus

NAOYA NIIMI,[1,2] WORAWIDH WAJJWALKU,[3] YOSHIHIRO ANDO,[2,4] NOBUHISA NAKAMURA,[2] MINORU UEDA,[1] AND YASUNOBU YOSHIKAI[2]*

Department of Oral Surgery,[1] Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control,[2] and Department of Pediatrics,[4] Nagoya University School of Medicine, Nagoya, Japan, and Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Nakhonpathom, Thailand[3]

Received 15 May 1995/Accepted 9 August 1995

We have previously reported new *Mtv* loci, *Mtv-48* and *-51*, in the Japanese laboratory mouse strains CS and NC. Here we show by backcross analysis that both *Mtv-48* and *-51* cosegregate with very slow deletion of T cells bearing Vβ 2. The nucleotide sequences of the open reading frames in the 3′ long terminal repeats of *Mtv-48* and *-51* were very similar to those of *Mtv-DDO*, mouse mammary tumor virus C4 [MMTV(C4)], and MMTV(BALB/cV), which encode Vβ 2-specific superantigens. Furthermore, backcross female mice carrying *Mtv-48* but not *Mtv-51* were found to be able to produce milk-borne MMTV(CS), which can vigorously stimulate Vβ 2-expressing T cells after local injection in vivo in an I-E-dependent manner. On the other hand, mice carrying *Mtv-51* but not *Mtv-48* could not produce such an MMTV in milk. The nucleotide sequences of MMTV(CS) open reading frame were completely matched with those of *Mtv-48*. These results indicate that the provirus *Mtv-48* but not *Mtv-51* is capable of producing a milk-borne virus of which the superantigen stimulates Vβ 2-expressing T cells.

Mouse mammary tumor virus (MMTV), which belongs to a family of milk-borne type B retroviruses, is responsible for the induction and transmission of mammary carcinomas in mice (12). Endogenous mouse mammary tumor viruses (*Mtv* proviruses) are integrated in the germline of all inbred mouse strains and most wild mice (22). In almost all the inbred mouse strains, *Mtv* proviruses are scattered on different chromosomes and heterogeneously distributed from one strain to another. Recently, it has been proved that the open reading frame (ORF) in the 3′ long terminal repeat (LTR) of *Mtv* encodes a superantigen that leads to in vivo deletion and in vitro stimulation of T cells bearing particular Vβ gene products (2, 5, 17, 18). In vitro translation studies indicate that the *Mtv* ORF product is a type II transmembrane molecule in which the COOH terminus is extracellular (6, 19, 21), and comparison of amino acid sequences of *Mtv* ORFs and gene transfection experiments with chimeric *Mtv* ORFs reveal that the COOH terminus is responsible for the Vβ specificity of superantigens (2, 5, 34). Studies with transgenic mice show that absence of the superantigen-reactive T cells interrupts exogenous MMTV transmission from infected mothers to their offspring via milk, indicating a biological role of superantigens in infection with MMTV (9–11).

Almost all the endogenous *Mtv* proviruses have undergone mutations which cause defects in their biological activity and therefore cannot produce infectious (exogenous) MMTV (27). Only a few *Mtv* proviruses have been identified to be able to produce infectious MMTV. MMTV(GR) encoding Vβ 14-specific superantigen is thought to be derived from *Mtv-2* (4, 8, 24, 31). MMTV(SHN) is from *Mtv-4* and encodes deletion ligand for Vβ 7, 8.1-3 T cells (23).

We have previously reported new *Mtv* loci, *Mtv-48* and *-51*, in the Japanese laboratory mouse strains CS and NC (32, 33). The inbred CS ($H\text{-}2^b$) and NC strains were provided by the Institute for Laboratory Animal Research, Nagoya University School of Medicine. The CS strain was established by crossbreeding of the S-II strain with the NBC strain (20, 29), which carries *Mtv-3*, *-6*, *-8*, *-17*, *-46*, *-48*, *-49*, *-50*, and *-51* (32, 33). The NC strain was established by crossbreeding with strains of Japanese pet mouse origin (7, 14, 26) carrying *Mtv-3*, *-8*, *-13*, *-17*, *-48*, *-50*, and *-51* (33). In the present study, we have found by backcross analysis that the very slow deletion of T cells bearing Vβ 2 cosegregates with both *Mtv-48* and *-51*. The COOH termini of *Mtv-48* and *-51* are very similar to those of *Mtv-DDO*, MMTV(C4), and MMTV(BALB/cV), which can encode Vβ 2-specific superantigens (13, 15, 16, 28). Furthermore, we have found that milk from backcross mice carrying *Mtv-48* but not those carrying *Mtv-51* contains milk-borne MMTV(CS) which can vigorously stimulate Vβ 2-expressing T cells after local injection in vivo in an I-E-dependent manner. Comparison with ORF sequences of *Mtv-48* and MMTV(CS) strongly suggested that *Mtv-48* should produce a milk-borne MMTV.

To address the question of whether *Mtv-48* and *Mtv-51* encode deletion ligands for certain Vβ-expressing T cells, we first examined the Vβ repertoire in $CD4^+$ lymph node (LN) cells in $(BALB/c \times NC)F_1$ and $(BALB/c \times CS)F_1$ mice by flow cytometric analysis. LN cells or peripheral blood lymphocytes (10^6) were stained in one step with a mixture of fluorescein isothiocyanate-labeled anti-TcR Vβ antibody and phycoerythrin-conjugated anti-CD4. All samples were analyzed on a FACScan cell sorter (Becton Dickinson, Mountain View, Calif.) by using Lysis II software. Dead cells were excluded by means of forward and side scatter. As shown in Fig. 1, because of the presence of *Mtv-6*, *-8*, and *-9* derived from BALB/c and *Mtv-50*

* Corresponding author. Mailing address: Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466, Japan. Phone: 81-52-741-2111, ext. 2102. Fax: 81-52-731-9479. Electronic mail address: yyoshika@tsuru.med.nagoya-u.ac.jp.

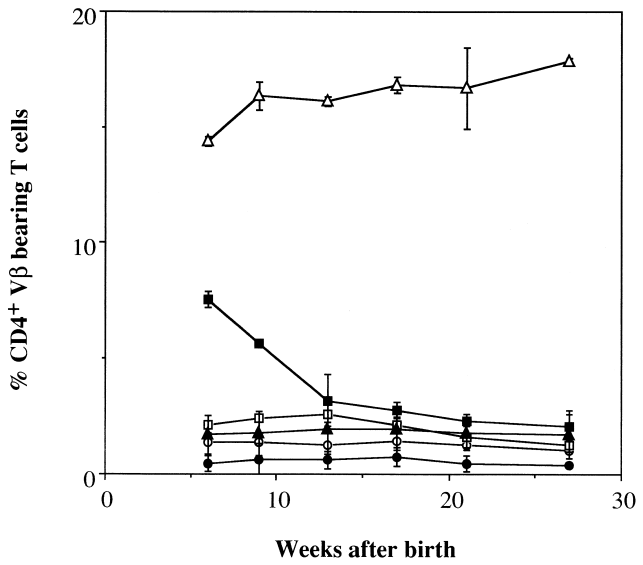

FIG. 1. Kinetics of Vβ repertoire of peripheral $CD4^+$ T cells in (BALB/c × CS)F_1 mice. T cells were isolated from the LN of (BALB/c × CS)F_1 mice at the indicated time points after birth and tested for TcR Vβ expression among $CD4^+$ T cells by using a FACScan cell sorter (Becton Dickinson). Shaded squares indicate the proportion of Vβ 2^+ T cells in $CD4^+$ T cells, open squares indicate that of Vβ 3^+ T cells, shaded circles indicate that of Vβ 5^+ T cells, open circles indicate that of Vβ 6^+ T cells, shaded triangles indicate that of Vβ 11^+ T cells, and open triangles indicate that of Vβ 14^+ T cells. The data are expressed as means and standard deviations (error bars) for three mice.

derived from NC or CS, which encode deletion ligands for Vβ 3, Vβ 5, Vβ 11, or Vβ 6^+ $CD4^+$ T cells, these T cells were deleted in the periphery well before mice were 6 weeks old. The proportion of Vβ 2^+ $CD4^+$ T cells did not change until the mice reached about 10 weeks of age, but the clonal deletion of Vβ 2^+ $CD4^+$ T cells was nearly completed by the time mice were 6 months of age. On the contrary, numbers of Vβ 14^+ $CD4^+$ T cells increased gradually as a result of compensation. Thus, Vβ 2^+ $CD4^+$ T cells were found to be deleted, albeit with very slow kinetics, in (BALB/c × NC)F_1 and (BALB/c × CS)F_1 mice.

Next we examined the Vβ repertoire in $CD4^+$ LN cells in BALB/c × (BALB/c × NC)F_1 backcross mice (B×BNC) and BALB/c × (BALB/c × CS)F_1 backcross mice (B×BCS) >6 months old by flow cytometric analysis. *Mtv-48* was detected as a 3.4-kb *Eco*RI fragment hybridizing with an MMTV envelope probe, and *Mtv-51* was detected as a 5.8-kb *Pvu*II fragment hybridizing with an MMTV LTR probe (Fig. 2). Representative data on flow cytometric analysis of peripheral lymphocytes of B×BNC and B×BCS are shown in Table 1 and Table 2, respectively. Of 57 mice, 25 mice carrying *Mtv-48* or *Mtv-51* had low levels of Vβ 2^+ T cells (0.24 to 3.77%), whereas 32 mice carrying neither *Mtv-48* nor *Mtv-51* had high levels of Vβ 2^+ T cells (5.88 to 16.74%). Thus, there was a cosegregation between mice having low levels of Vβ 2^+ $CD4^+$ T cells and those carrying *Mtv-48* or *Mtv-51*, while mice having high levels of Vβ 2^+ $CD4^+$ T cells carried neither *Mtv-48* nor *Mtv-51*. Taken together, these results suggest that both *Mtv-48* and *Mtv-51* govern the deletion of T cells bearing Vβ 2, similar to the situation with *Mtv-DDO* (15).

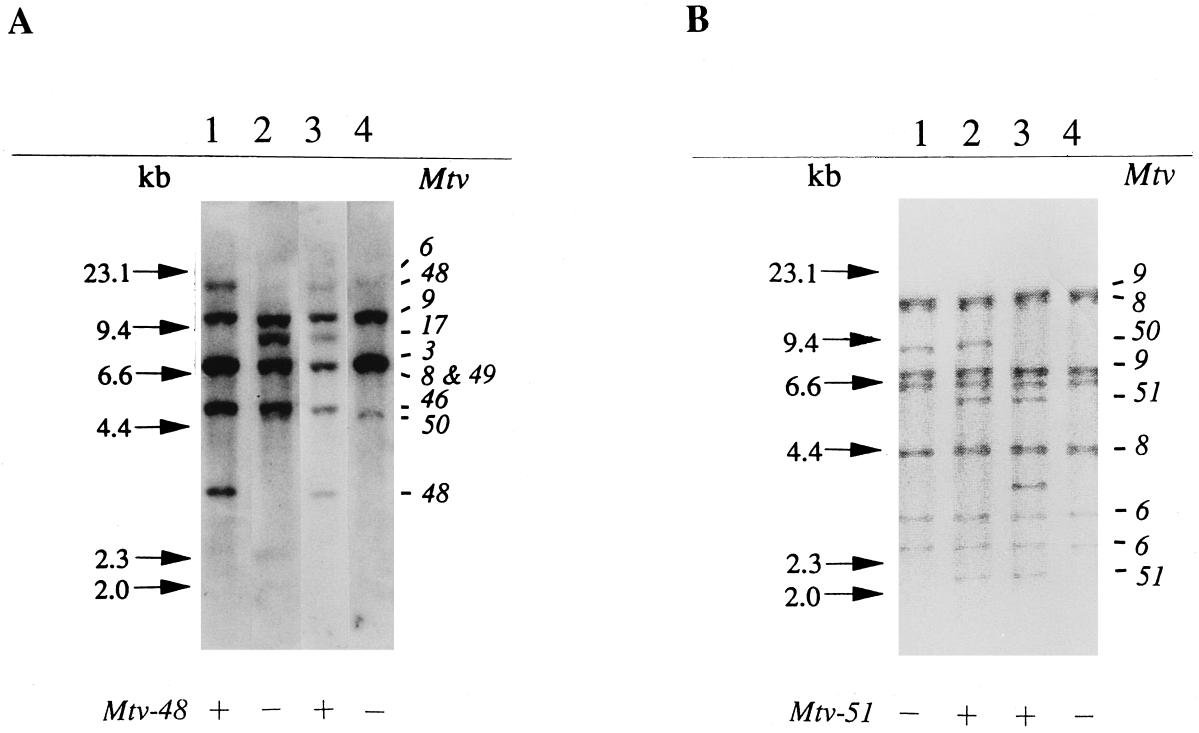

FIG. 2. Southern blot analysis of *Mtv-48* and *Mtv-51* segregation in B×BCS mice. The DNA (10 μg) was digested with *Eco*RI or *Pvu*II, separated by electrophoresis in a 0.5% agarose gel, and then transferred to GeneScreen Plus membranes (Dupont, Boston, Mass.). The membranes were hybridized with a ^{32}P-labeled *env* or LTR probe in hybridization buffer containing 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate, 100 μg of salmon sperm DNA per ml, and 3× Denhardt's solution at 45°C overnight. Bound probe was detected by using the Fujix BAS 2000 Image Analyzing System (Fuji Photo Film Co., Tokyo, Japan). (A) *Eco*RI-digested DNA, hybridized with *env* probe; (B) *Pvu*II-digested DNA, hybridized with LTR probe.

TABLE 1. Linkage between endogenous *Mtv* loci and TcR Vβ repertoire in B×BNC mice

Mouse strain	*Mtv* locus[a]		% Indicated Vβ in $CD4^+$ T cells[b]	
	48	*51*	Vβ 2	Vβ 8.2
BALB/c	−	−	6.68 ± 0.58	13.81 ± 1.71
NC	+	+	**0.77 ± 0.21**	Gene deleted
BALB/c × NC	+	+	**0.55 ± 0.12**	9.66 ± 0.65
B×BNC 2	+	+	**0.68**	11.03
B×BNC 3	−	−	5.88	14.45
B×BNC 4	−	−	7.98	13.78
B×BNC 5	−	−	7.72	16.87
B×BNC 6	+	+	**1.31**	12.49
B×BNC 7	+	+	**0.24**	15.11
B×BNC 8	−	+	**1.23**	18.41
B×BNC 10	+	−	**1.31**	16.11
B×BNC 11	+	−	**1.34**	11.98
B×BNC 13	+	−	**1.22**	12.46
B×BNC 15	+	−	**0.97**	11.06
B×BNC 16	+	−	**1.28**	12.67
B×BNC 17	+	−	**0.97**	11.69

[a] −, absent; +, present.
[b] The data are expressed as means and standard deviations for five mice for BALB/c, NC, and BALB/c × NC mice. Other *Mtv* genotypes are described in reference 33. Boldface indicates significantly decreased percentages.

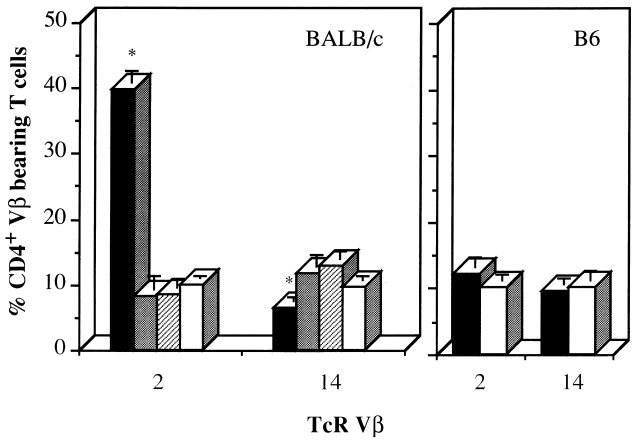

FIG. 3. Expansion of Vβ 2^+ $CD4^+$ T cells in adult BALB/c mice injected with MMTV(CS). Adult BALB/c or B6 mice were injected in the hind footpad with 30 μl of partially purified viruses or phosphate-buffered saline (PBS) as a control. Four days later, the popliteal LNs were isolated and analyzed with a FACScan cell sorter (Becton Dickinson). Shown are results for milk from *Mtv-48*-positive, *Mtv-51*-negative females (■); *Mtv-48*-negative, *Mtv-51*-positive females (▨); and *Mtv-48*-negative, *Mtv-51*-negative females (▨) and PBS as a control (□). The data are expressed as means and standard deviations (error bars) for three mice. The statistical significance of the data was determined by the paired Student's *t* test. An asterisk indicates significant difference from the control value ($P < 0.01$).

The injection of milk into adult mice enables the direct monitoring of events that follow virus challenge. Within a few days of injection of milk into the footpad, a proliferative response of the superantigen-reactive $CD4^+$ T cells occurs in the draining LN, followed by selective deletion of the relevant $CD4^+$ T cells in the peripheral blood (1). We bred B×BCS and collected milk from *Mtv-48*- or *Mtv-51*-positive females or *Mtv-48*- or *Mtv-51*-negative females previously described (28). Purified viruses from milk of B×BCS mice were injected into the hind footpads of adult BALB/c ($H\text{-}2^d$) and B6 ($H\text{-}2^b$) mice. Four days later, vigorous expansion of Vβ 2^+ $CD4^+$ T cells in the popliteal LN in BALB/c mice was evident when B×BCS milk from *Mtv-48*-positive females was injected ($P < 0.01$). In contrast, such an increase was not evident when milk from *Mtv-51*-positive but *Mtv-48*-negative females was injected (Fig. 3). On the other hand, in B6 mice Vβ 2^+ $CD4^+$ T cells never expanded after injection of mice with milk from *Mtv-48*-positive females. Vβ 2^+ $CD4^+$ T cells were selectively deleted over 2 weeks in the peripheral blood of BALB/c mice injected with the viruses. In contrast, the proportion of Vβ 2^+ $CD4^+$ T cells did not alter after mice were injected with milk from *Mtv-51*-positive but *Mtv-48*-negative females. Deletion of Vβ 2^+ T cells did not occur in B6 mice, even after injection with milk from *Mtv-48*-positive females (data not shown). Thus, it would appear that only mice carrying *Mtv-48* can release Vβ 2-specific exogenous MMTV into the milk and that mice carrying

TABLE 2. Linkage between endogenous *Mtv* loci and TcR Vβ repertoire in B×BCS mice

Mouse strain	*Mtv* locus[a]										% Indicated Vβ in $CD4^+$ T cells[b]	
	3	*6*	*8*	*9*	*17*	*46*	*48*	*49*	*50*	*51*	Vβ 2	Vβ 14
BALB/c	−	+	+	+	−	−	−	−	−	−	6.68 ± 0.58	10.32 ± 0.22
CS ($I\text{-}E^-$)	+	+	+	−	+	+	+	+	+	+	9.14 ± 0.24	16.98 ± 0.12
BALB/c × CS	+	+	+	+	+	+	+	+	+	+	**2.07 ± 0.54**	16.89 ± 0.07
B×BCS 1	+	+	+	+	+	−	+	−	+	−	**2.01**	15.08
B×BCS 2	−	+	+	+	−	−	−	−	+	+	**1.94**	16.22
B×BCS 3	−	+	+	+	−	+	−	+	+	−	15.36	15.66
B×BCS 4	+	+	+	+	−	−	−	+	+	+	**1.02**	16.16
B×BCS 5	−	+	+	+	−	+	+	−	−	+	**3.15**	16.73
B×BCS 6	+	+	+	+	+	−	−	+	−	−	14.36	8.02
B×BCS 7	+	+	+	+	−	+	−	−	−	−	16.74	12.59
B×BCS 8	−	+	+	+	−	−	−	−	−	+	**2.45**	9.65
B×BCS 9	+	+	+	+	−	+	−	+	+	+	**3.77**	13.12
B×BCS 10	−	+	+	+	+	+	−	−	−	−	15.30	13.26
B×BCS 11	−	+	+	+	+	−	−	+	−	−	11.40	8.83
B×BCS 12	+	+	+	+	−	−	−	+	−	−	11.06	8.71
B×BCS 13	−	+	+	+	+	+	+	−	−	−	**2.37**	13.79
B×BCS 14	+	+	+	+	−	+	−	−	−	−	15.70	8.11
B×BCS 15	−	+	+	+	−	+	−	+	−	−	12.75	9.49

[a] −, absent; +, present.
[b] The data are expressed as means and standard deviations for three to five mice for BALB/c, CS, and BALB/c × CS mice. Boldface indicates significantly decreased percentages.

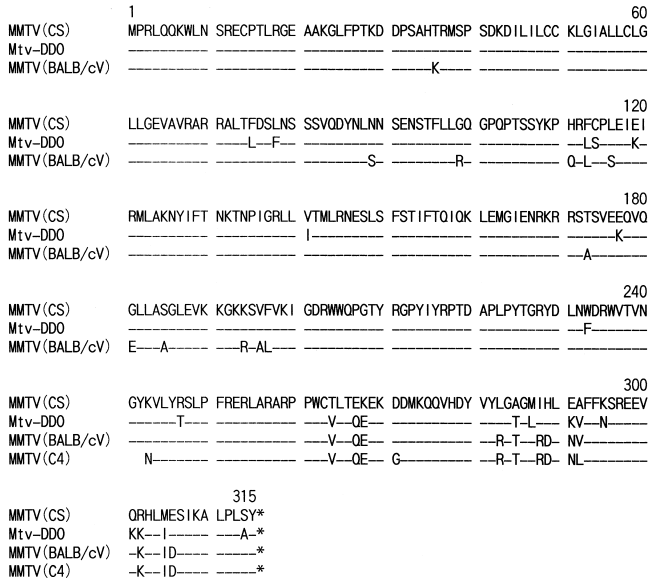

FIG. 4. Comparison of predicted amino acid sequence of MMTV(CS) ORF with known sequences of ORFs which encode Vβ 2-specific superantigens. Identical amino acids are represented by dashes; asterisks indicate the termination codon.

only *Mtv-51* do not release Vβ 2-specific exogenous MMTV into the milk. Furthermore, it is suggested that Vβ 2-specific superantigen encoded by *Mtv-48* is strictly dependent on I-E molecules for superantigen activity, unlike superantigen encoded by *Mtv-DDO* (15).

In order to confirm that Vβ 2-specific exogenous MMTV in the milk is derived from endogenous *Mtv-48*, we compared the nucleotide sequences of the ORFs of *Mtv-48* and *Mtv-51* with those of milk-derived virus from B×BCS [MMTV(CS)]. The genomic DNA was digested with *Eco*RI or *Pvu*II. The *Eco*RI-digested 3.4-kb fragment corresponding in size to the *Mtv-48* ORF and the *Pvu*II-digested 5.8-kb fragment corresponding in size to the *Mtv-51* ORF were isolated on a low-melting-point agarose gel (33). The DNAs were amplified by PCR with the 5′ oligonucleotide *Mtv* common primer ATGCCGCGCCTGCA GCAGA (positions 1 to 19) and the 3′ oligonucleotide *Mtv* common primer AAGTCAGGAAACCACTTGT (positions 1061 to 1080), selected from the ORF conserved region (3). On the other hand, the cDNA from milk-derived MMTV was amplified with common *Mtv* LTR sense and antisense primers in 1× PCR buffer under the PCR conditions of 94°C for 1 min, 45°C for 2 min, and 72°C for 3 min for 30 cycles. The PCR products were size fractionated in 1% agarose gels, cloned into the pCR II vector (TA cloning kit; Invitrogen Co., San Diego, Calif.), and sequenced by using the *Taq* dye primer cycle sequencing kit (Applied Biosystems Inc., Foster City, Calif.). The nucleotide sequences of *Mtv-48*, *Mtv-51*, and MMTV(CS) are available from EMBL, GenBank, and DDBJ under accession number D49536. Sequence comparison revealed that the *Mtv-48* ORF was completely identical to the MMTV(CS) ORF. However, the *Mtv-51* ORF has a 2-nucleotide replacement. The peptide amino acid sequences of MMTV(CS) were compared with those of other *Mtv* ORF products specific for Vβ 2. As shown in Fig. 4, in the region of the COOH terminus the MMTV(CS) ORF product has a high degree of homology to sequences of recently reported *Mtv-DDO*, MMTV(C4), MMTV(C3H-K), and MMTV(BALB/cV) ORF products (15, 16, 28). Over a stretch of 20 amino acids, there were only a few substitutions. On the other hand, the COOH terminus was very divergent from other *Mtv* ORF sequences previously published (data not shown) (30). The COOH terminus of these Vβ 2-specific MMTV ORF sequences apparently helps to determine the key residues for TcR Vβ.

Many of the endogenous *Mtv* proviruses, as well as exogenous MMTV, require major histocompatibility complex class II I-E expression for clonal deletion and stimulation of their superantigen-reactive T cells. The MMTV(CS) strain we describe here strictly requires I-E expression for clonal deletion and stimulation, as do MMTV(C3H) and MMTV(GR) (1, 8). On the other hand, *Mtv-DDO*, MMTV(BALB/cV), and MMTV(C4) do not depend on I-E expression for clonal deletion and stimulation (13, 15, 28). *Mtv-DDO* induced vigorous stimulation and fast kinetics of clonal deletion, whereas MMTV(CS) induced vigorous stimulation and very slow kinetics of clonal deletion. The difference in kinetics of clonal deletion of Vβ 2^+ T cells in mice carrying *Mtv-48* or *Mtv-51* and in mice carrying *Mtv-DDO* may be partially explained by their different I-E dependencies. Previous reports have proposed that strong superantigens do not depend on major histocompatibility complex class II I-E expression for clonal deletion and stimulation and that kinetics of clonal deletion are correlated with the extent of superantigen activity (1). However, MMTV(CS) showed a strong superantigen activity with very slow kinetics of clonal deletion in an I-E-dependent manner, like MMTV(SHN), which showed a strong superantigen activity with slow kinetics of clonal deletion in an I-E-independent manner (23). Therefore, the extent of superantigen activity may not be correlated with I-E dependency or kinetics of clonal deletion. We have previously reported that ontogenical expression of the *Mtv* ORF in the thymus differs among *Mtv* proviruses (25). Therefore, the quantities of the superantigens expressed in the thymus and in the periphery may be differently regulated in *Mtv-48*, *Mtv-51*, and *Mtv-DDO* mice.

MMTV is transmitted from infected mothers to their offspring via milk or by Mendel's law as an endogenous *Mtv* which is integrated in the germline (27). Almost all the endogenous *Mtv* proviruses have undergone mutations which resulted in loss of the ability to produce infectious viral particles (27). Comparison of the two complete *Mtv* sequences may provide a clue to the cause of the different biological activities of *Mtv-48* and *-51* in the production of a milk-borne MMTV.

In conclusion, we have identified new *Mtv-48* and *Mtv-51* loci encoding a deletion ligand for Vβ 2. *Mtv-48* but not *Mtv-51* can produce milk-borne MMTV(CS), which vigorously stimulates Vβ 2^+ T cells after local injection in vivo in an I-E-dependent manner.

We especially thank B. Malissen, H. Hengartner, P. Marrack, and J. Kappler for providing the monoclonal antibodies (B20.6, 44-22-1, and F23.2) and A. Murakami for providing MMTV probes. We also thank H. W. Borgeld for critical comments on the manuscript.

This work was supported in part by grants to Y.Y. from the Ministry of Education, Science and Culture; the Ministry of Health and Welfare; and Funds of the Science and Technology Agency of the Japanese Government.

REFERENCES

1. **Acha-Orbea, H., and E. D. Palmer.** 1991. Mls—a retrovirus exploits the immune system. Immunol. Today **12:**356–361.
2. **Acha-Orbea, H., A. N. Shakhov, L. Scrapellino, E. Kolb, V. Müller, A. Vessaz-Shaw, R. Fuchs, K. Blöchlinger, P. Rollini, J. Billote, M. Sarafidou, H. R. MacDonald, and H. Diggermann.** 1991. Clonal deletion of Vβ14 positive T cells in mammary tumor virus transgenic mice. Nature (London) **350:**207–211.
3. **Brandt-Carlson, C., J. S. Butel, and D. Wheeler.** 1993. Phylogenic and structural analyses of MMTV LTR ORF sequences of exogenous and endogenous origins. Virology **193:**171–185.

4. **Cho, K., D. A. Ferrick, and D. W. Morris.** 1995. Structure and biological activity of the subgenomic Mtv-6 endogenous provirus. Virology **206:**395–402.
5. **Choi, Y., J. W. Kappler, and P. Marrack.** 1991. A superantigen encoded in the open reading frame of 3′ long terminal repeat of mouse mammary tumor virus. Nature (London) **350:**203–207.
6. **Choi, Y., P. Marrack, and J. W. Kappler.** 1992. Structural analysis of a mouse mammary tumor virus superantigen. J. Exp. Med. **175:**847–852.
7. **Eisenberg, R. A., A. N. Theofilopolous, B. S. Andrews, C. J. Peters, L. Thor, and F. J. Dixon.** 1979. Natural thymocytotoxic autoantibodies in autoimmune and normal mice. J. Immunol. **122:**2272–2278.
8. **Ferrick, D. A., K. Cho, L. Gemmell-Hori, and D. W. Morris.** 1992. Genetic analysis of the effects of *Mtv-2* on the T cell repertoire in the WXG-2 mouse strain. Int. Immunol. **4:**805–810.
9. **Golovkina, T. V., A. Chervonsky, J. P. Dudley, and S. R. Ross.** 1992. Transgenic mouse mammary tumor virus superantigen expression prevents viral infection. Cell **69:**637–645.
10. **Golovkina, T. V., J. A. Prescott, and S. R. Ross.** 1993. Mouse mammary tumor virus-induced tumorigenesis in *sag* transgenic mice: a laboratory model of natural selection. J. Virol. **67:**7690–7694.
11. **Held, W., G. A. Waanders, A. N. Shakhov, L. Scarpellino, H. Acha-Orbea, and H. R. MacDonald.** 1993. Superantigen-induced immune stimulation amplifies mouse mammary tumor virus infection and allows virus transmission. Cell **74:**529–540.
12. **Heston, W. E., M. K. Deringer, and H. B. Andervont.** 1945. Gene-milk agent relationship in mammary tumor development. J. Natl. Cancer Inst. **5:**289–307.
13. **Hodes, R. J., M. B. Novick, L. D. Palmer, and J. E. Knepper.** 1993. Association of a Vβ2-specific superantigen with a tumorigenic milk-borne mouse mammary tumor virus. J. Immunol. **150:**1422–1428.
14. **Itoh, K., T. Oowada, and T. Mitsuoka.** 1985. Characteristic faecal flora of NC mice. Lab. Anim. **19:**7–15.
15. **Jouvin-Marche, E., P. N. Marche, A. Six, C. Liebe-Gris, D. Voegtle, and P.-A. Cazenave.** 1993. Identification of an endogenous mammary tumor virus involved in the clonal deletion of Vβ 2 T cells. Eur. J. Immunol. **23:**2758–2764.
16. **Kang, J. J., T. Schwegel, and J. E. Knepper.** 1993. Sequence similarity between the long terminal repeat coding regions of mammary-tumorigenic BALB/cV adrenal-tumorigenic C3H-K strains of mouse mammary tumor virus. Virology **196:**303–308.
17. **Kappler, J. W., N. Roehm, and P. Marrack.** 1987. T cell tolerance by clonal elimination in the thymus. Cell **49:**273–280.
18. **Kappler, J. W., U. D. Staerz, J. White, and P. C. Marrack.** 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature (London) **332:**35–40.
19. **Knight, A. M., G. B. Harrison, R. J. Pease, P. J. Robinson, and P. J. Dyson.** 1992. Biochemical analysis of the mouse mammary tumor virus long terminal repeat product. Evidence for the molecular structure of an endogenous superantigen. Eur. J. Immunol. **22:**879–882.
20. **Kondo, K., and K. Esaki.** 1962. Breeding of tester strains for coat colour genes. Bull. Exp. Anim. **11:**194–196.
21. **Korman, A. J., P. Bourgrel, T. Meo, and G. E. Rieckhof.** 1992. The mouse mammary tumor virus long terminal repeat encodes a type II transmembrane glycoprotein. EMBO J. **11:**1901–1905.
22. **Kozak, C., G. Peters, R. Pauley, V. Morris, R. Michalides, J. Dudley, M. Green, M. Davisson, O. Prakash, A. Vaidya, J. Hilgers, A. Verstraeten, N. Hynes, H. Diggelmann, D. Peterson, J. C. Cohen, C. Dickson, N. Sarkar, R. Nusse, H. Varmus, and R. Callahan.** 1987. A standardized nomenclature for endogenous mouse mammary tumor viruses. J. Virol. **61:**1651–1654.
23. **Luther, S., A. N. Shakhov, I. Xenarios, S. Haga, S. Imai, and H. Acha-Orbea.** 1994. New infectious mammary tumor virus superantigen with Vβ-specificity identical to staphylococcal enterotoxin B (SEB). Eur. J. Immunol. **24:**1757–1764.
24. **Morris, D. W., L. J. T. Young, M. B. Gardner, and R. D. Cardiff.** 1986. Transfer, by selective breeding, of the pathogenic *Mtv-2* endogenous provirus from the GR strain to a wild mouse line free of endogenous and exogenous mouse mammary tumor virus. J. Virol. **58:**247–252.
25. **Niimi, N., W. Wajjwalku, Y. Ando, S. Tomida, M. Takeuchi, M. Ueda, T. Kaneda, and Y. Yoshikai.** 1994. Delay in expression of a mammary tumor provirus is responsible for defective clonal deletion during postnatal period. Eur. J. Immunol. **24:**488–491.
26. **Nozaki, K., and K. Kondo.** 1956. Genetical study on a new type of crooked-tail in Japanese laboratory mice. Proc. Natl. Acad. Sci. USA **32:**293–297.
27. **Salmons, B., and W. H. Günzburg.** 1987. Current perspectives in the biology of mouse mammary tumor virus. Virus Res. **8:**81–102.
28. **Shakhov, A. N., H. Wang, H. Acha-Orbea, R. J. Pauley, and W.-Z. Wei.** 1993. A new infectious mammary tumor virus in the milk of mice implanted with C4 hyperplastic alveolar nodules. Eur. J. Immunol. **23:**2765–2769.
29. **Staats, J.** 1980. Standardized nomenclature for inbred strains of mice: seventh listing. Cancer Res. **40:**2083–2128.
30. **Tomonari, K., S. Fairchild, and O. A. Rosenwasser.** 1993. Influence of viral superantigens on Vβ- and Vα-specific positive and negative selection. Immunol. Rev. **131:**131–168.
31. **van Nie, R., A. A. Verstaeten, and J. de Moes.** 1977. Genetic transmission of mammary tumor virus by GR mice. Int. J. Cancer **19:**383–390.
32. **Wajjwalku, W., M. Takahashi, O. Miyaishi, J. Lu, K. Sakata, T. Yokoi, S. Saga, M. Imai, M. Matsuyama, and M. Hoshino.** 1991. Tissue distribution of mouse mammary tumor virus (MMTV) antigens and new endogenous MMTV loci in Japanese laboratory mouse strain. Jpn. J. Cancer Res. **82:** 1413–1420.
33. **Wajjwalku, W., S. Tomida, M. Takahashi, M. Matuyama, and Y. Yoshikai.** 1993. A gene encoding ligand for deletion of T cells bearing TcrVβ6 and Vβ8.1 cosegregate with a new endogenous mouse mammary tumor virus. Immunogenetics **37:**397–400.
34. **Yazdanbakhsh, K., C. G. Park, G. M. Winslow, and Y. Choi.** 1993. Direct evidence for the role of COOH terminal of mouse mammary tumor virus superantigen in determining T cell receptor Vβ specificity. J. Exp. Med. **178:**737–741.